PRELIMINARY STUDY OF TRACE ELEMENTS IN HUMAN BRAIN TUMOUR TISSUES BY INAA

Zhuang Guisun (庄圭荪), Wang Yinsong (王荫淞), Tan Mingguang (谈明光)
Zhi Min (支 敏), Wang Yongji (王永吉) and Pan Weiqing (潘伟清)
(Shanghai Institute of Nuclear Research, Academia Sinica, Shanghai 201800, China)
Zhang Fulin (张福林)
(Departments of Pathology and Neurology, Huashan Hospital, Shanghai 200040, China)

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ABSTRACT

Elemental profiles of brain tumour tissues from 15 male patients of astrocytomas (grade I- III) and normal human brain tissues of 23 male age matched autopsies as controls have been studied by INAA. A total of 18 elements Se, Na, K, Br, Cl, Mn, Mg. S, Ca, Cu, Hg, Cr, Fe, Rb, Zn, Co, Sc and P has been determined for this purpose. The analytical results showed that compared with the normal brain tissues, concentrations of Ca, Fe, Cu, Zn, Mn, Br and Sc were significantly higher in tumour tissues and that of Rb, K and P were lower while no differences for contents of Mg, S, Cr, Na and Cl were observed. A negative correlation between P and Ca in malignant and normal brain tissues was observed.

Keywords: Trace element Brain cancer NAA

I. INTRODUCTION

There has been a growing research interest in the role of metals on human health and disease, as the environment gets polluted. The central nervous system, which is sensitive to trace element disturbances because of its high metabolic rate, is of great concern. Study on trace element regional distributions in animal and normal human brain have been reported[1-5]. malignant brain tumours, however, are so far incurable and it is important to study the tumours on basis of elemental compositions and their characteristics. The most effective way to study this problem is to analyze as many elements as possible in a single sample and to collect as many samples as possible. PIXE analysis has been used for the determination of 12 elements in human brain tumour tissues from 14 patients by Tapper[6] and 14 elements for one subject by Wei[7]. However, the differences of element levels between normal and tumour, the relationship between elements in brain tumour tissues and variation of element contents in different stages of tumour development are still little known. That encouraged us to investigate the alternated concentrations of essential and nonessential elements in brain tumour tissues. Instrumental neutron activation analysis is an accurate and sensitive method for multielement analysis. The simplicity of sample preparation minimizes the risk of contamination. The details of INAA determination of trace elements in biological samples have been described else-

where[8-9].

Ⅱ. EXPERIMENTAL

1. Collection and pretreatment of samples

The test group consisted of 15 male malignant brain tumours (astrocytomas grade Ⅰ - Ⅲ) taken during neurosurgery at Huashan Hospital, Shanghai. The mean age was 45 years, ranging from 28 to 64. The controls are normal brain tissues from 23 age- matched male autopsy, who died from accident and proved to be in good health when alive. The cerebral cortical sections containing both grey and white mater were collected from the left hemisphere. To avoid external contamination, the surface of all tissue samples were cut off with titanium knife, diced into 1- 2 cm cubes in a clean bench and stored in a freezer at temperatures below - 30℃ .

All tissues were freeze dried for 48 h, homogenized by brittle fracture technique in liquid nitrogen and ground into fine powder. The ratio of freeze- dry to wet weight were determined for each sample.

2. Irradiation and counting

Samples of 100mg dry weight were double sealed into polyethylene envelops for irradiation. The chemical standards were prepared with appropriate solution on clean filter paper and sealed in a polyethylene envelope, too. The samples and standards were successively placed in a pneumatic transfer rabbit system and irradiated at a thermal neutron flux of $5- 8\times 10^{11}$ $n.cm^{-2}s^{-1}$ for 60- 400 s in MNSR reactor and a thermal neutron flux of $1\times 10^{13} n.cm^{-2}s^{-1}$ for 50 h in swimming pool reactor for short and long- lived radioisotope analysis, respectively. The activities of nuclides were measured by an Ortec Ge (Li) detector with a resolution of 1.9 keV for 1332 keV γ - ray. The detector was coupled to a CANBEERA S- 80 4096 channel pulse height analyzer interfaced with a PDP 11/34 computer system. The net peak area, statistic error and element contents in ppm were determined automatically by a SPAN code.

Ⅲ. RESULTS AND DISCUSSION

To check up the reliability of the procedure, biological standard reference materials, Horse kidney (IAEA H- 8) and Bovine Liver (NBB SRM 1577a), were analyzed. The analytical values agreed with the NBS and IAEA certified values. The relative standard deviation for most of these elements was found to be less than 5- 10% (Tab.1). The analytical results of element concentrations in normal brain and brain tunour tissues with comparisons of the literature values were given in Tab.2.

The analytical results presented in this study for most element contents were in close agreement with the results presented by Abdulla for normal brain tissues and by Tapper and Wei[6,7] for malignant brain tissues. With the exception of S, Mg, Cr and Co, significant differences for element contents between normal and malignant brain

tissues were found. The causes of these differences remain to be clarified, but we might be able to discuss on the roles of some of the elements.

Table 1

The analytical results for standard reference materials (mg/kg)

Element	NBS bovin	Liver (1577a)	IAEA horse	Kidney (H- 8)
	This work	NBS	This work	IAEA
Se	0.67±0.03	0.71±0.07	4.83±0.48	4.67±1.0
Cu	151.6±3.0	158±6.9	30.6±0.2	31.3±1.8
S(%)	0.77±0.02	0.78±0.09	0.956±0.001	0.95±0.15
Mn	9.75±0.11	9.9±0.80	5.64±1.52	5.73±0.28
Na	2341±51	2430±129	8827±418	9600±298
Ca	135±49	120±7	890±22.7	920±203
Cl	2611±39	2800±98	10800±960	12600±1058
Br	9.48±0.59	(9)	103±8.4	104±11.4
Mg	604±35	600±135	859±16	818±91
K	10950±938	9960±70	11430±1445	11700±749
Hg		0.004±0.002	0.88±0.13	0.91±0.08
Rb	12.1±0.8	12.5±0.1	21.6±0.8	22.2±0.8
Zn	120.3±4.6	123±8	191.1±8	193±6.0
Co	0.27±0.01	0.21±0.05	0.144±0.04	(0.13)
P	12109±513	11100±400	11548±170	11200±605

Table 2

The analytical results of measured elemental concentrations in normal human brain and brain tumour tissues and comparison with the literature values (g/g dry weight)

Element	Present work		Papper et al.[6]		C.C.Wei[7]	
	Tumour	Normal	Tumour	Normal	Tumour	Normal
	$n=15$	$n=23$	$n=7$	$n=64$		
P	9580±1900	14420±400	4600±800	10900±1400		
S	9470±3970	9830±3700	6000±700	6000±500		
K	4900±2200	13100±1500	7600±5600	13700±3200	433±20.8	893±29.9
Ca	1640±1200	241±150	620±210	350±70		
Mn	6.29±10.8	1.16±0.43	1.2±1.4	1.7±0.22		
Fe	539±402	224±35	340±140	260±110	71.7±4.5	17.3±2.1
Zn	82.8±21.9	41.3±5.6	67±14	54±20	30.3±5.5	8.0±2.8
Cu	38.5±22.5	20.2±3.7	12±7.0	19±4.5	23.4±3.1	29.8±2.5
Se	1.12±0.22	0.57±0.17	0.78±0.15	0.57±0.28	trace	3.6±1.7
Rb	11.2±6.3	22.2±7.0	10±12	17±7.1		
Cl	5400±2760	6420±831	6420±831			
Na	6165±2390	6160±733				
Mg	677±177	700±101				
Hg	0.24±0.22	0.17±0.08				
Cr	0.58±0.56	0.80±0.53				
Sc (ng/g)	6.7±6.2	1.67±0.95				
Co (ng/g)	62±8	35±6.3				
Br	7.48±3.13	3.27±1.16			6.7±1.6	51.1±7.1

Monovalent elements The electrolytes, i.e. the elemental concentrations of Cl

and Na, kept constant for the 23 normal human brain tissues (Na: 5– 6×10^3, Cl: 5– 8×10^3ppm). However, the variability for the 15 malignant brain tissues (Na: 3– 11×10^3, Cl: 2.3– 12.6×10^3ppm), although no significant differences for the mean values between the normal and tumour brain tissues could be found. Compared with the normal tissue, the Rb and K concentration in tumour tissues were decreased while the contents of Br elevated by 230% and it elevated with the malignant extent (Fig.1). The higher Br levels in the most necrotic cystic astrocytoma samples seems to reflect the ions through the cell membrane.

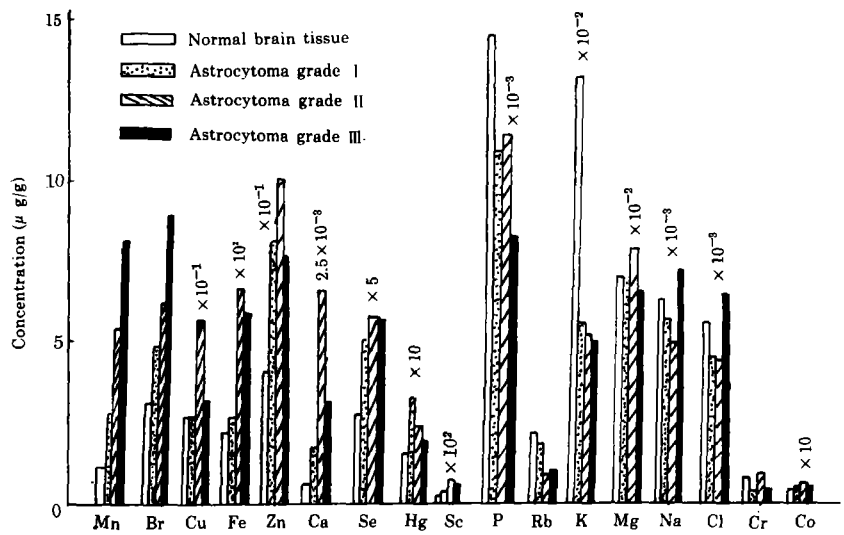

Fig.1 A comparison of trace element distribution between normal brain and brain tumour tissues with different malignant extent (astrocytoma grade (I—III)

Zinc The contents of Zn, Fe, Mn, Ca and Cu in the malignant brain tissues shoued a significant elevation compared with the controls. Zn is required for growth, development, reproduction and other important physiological functions. The higher concentration of Zn in the tumour tissues suggested that the element Zn was also a necessary part for growth of brain tumours.

Iron The brain tumour iron levels were significant higher ($P<0.01$) than that of normal tissues and the levels increased with the stage of malignant disease. The Fe contents in brain tumour tissues for grade II and III were twice for grade I and three times higher compared with the normal tissues. It may be related to increasing blood vessel specifically for astrocytoma and growth of tumour cell to cause a systemic Fe deficiency for brain tumour patients.

Calcium, copper and phosphorus The contents of calcium are in a constant range for normal brain tissues in this study. However high levels of Ca in tumour tissues were observed. The highest contents of Ca reached more than 4000 ppm, in tumour

tissues from two patients. It was interesting to find a positive correlation between Ca and Cu from statistic analysis for 15 subjects of tumour tissue (correlation coefficient r= 0.62, $P<0.01$) and a negative correlation between Ca and P for normal and tumour tissues (Fig.2) (r=– 0.83, $P<0.01$). Copper is a constituent of certain enzymes and is essential in their activities. Elevation of copper content for most tumour tissues was observed. The elevation of copper may indicate that copper is a main constituent of hemocuprein promoting the absorption, translation and ultilization of iron for the tumour growth. Phosphorus primarily as phospholipids is present in relatively high concentrations in brain. The phospholipid is the structural constituent of cell membrane. If the decrease of phosphorus in brain tumour reflects the alternation of function of cell membrane and if the negative correlation between Ca and P shows the element Ca is antagonistic to P in normal or tumour tissues and if supplement of phosphorus may be advantageous for preventing from brain tumour risk. That remain to be investigated.

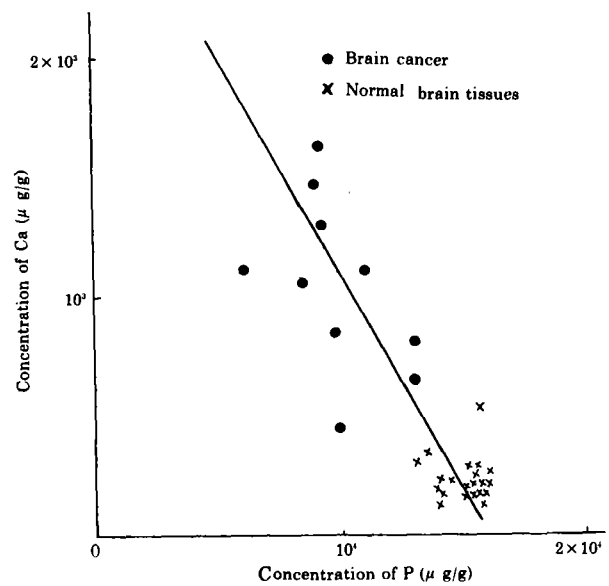

Fig.2 Correlation between Ca and P in brain cancer and normal brain tissues

Manganese Manganes is highly essential for the maintenance of reproductive function and glucose and lipid metabolism. Mn was accumulated in malignant brain tissues and increased with the malignant degree. It shows that the adsorption of Mn may be necessary for rapid growth and development of tumour tissues.

Mercury Mercury and methylmercury are the environmental pollutant that concerns many biological scientists because of their toxicity on brain damage and other central system disorders[11]. We found that Hg levels were slight higher in brain tumour tissues than in normal tissues. However, no significant variations of Hg levels

with the malignant degree were observed, it might not cause critical effects on tumour growth.

Selenium The specific role of Se in brain is not known. As a component of glutathione peroxidase it is important in protecting cell membranes from lipid peroxidation. Current evidence suggests that improved selenium nutrition can reduce cancer risk[12]. Compared with the normal tissue, an elevation of Se contents in brain tumour tissues was found. It was consisted with the results reported by Tapper[6]. No significant variations of Se contents with the malignant degree were observed. Se- antagonistic metal contents Hg, Cu, Fe and Zn were simultaneously increased in the malignant tissues might abolish the cancer- protecting effects of selenium.

Scandium Sc as a non- essential element is little known about its physiological function on the brain. Only ppb levels of Sc were presented in both normal brain and tumour, although a significant elevation of Sc level in tumour tissue, compared with normal brain, was observed. The effects of Sc for the growth and development on brain tumour are worth further investigating.

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